REMARKS

The new claims presented herein are supported by the disclosure as indicated in the following annotated version of the claims:

ANNOTATED VERSION OF NEW CLAIMS SHOWING SUPPORT THEREOF

65. (New) A microRNA oligonucleotide comprising:

a microRNA nucleotide sequence having a length which is at least 21 nucleotides long and is not longer than 23 nucleotides long; **[Fig. 13, paragraph 248: "a short ~22nt RNA segment"]**.

said microRNA nucleotide sequence forming part of a microRNA precursor nucleotide sequence, having a length which is greater than 60 nucleotides, said microRNA precursor nucleotide sequence being further characterized in that: **[Fig. 9, paragraph 230: "a minimum threshold for hairpin structure length"; Program Listing (file: HAIRPIN_PREDICTION.txt, functions: run_palgrade, get_palgrade, get_this_grade, get_nobulge_score; parameters: model_params); and Sequence Listing.]**

said microRNA precursor nucleotide sequence forms a hairpin structure, **[Fig. 9, paragraph 203: "HAIRPIN DETECTOR 114 operative to bioinformatically detect genomic `hairpin-shaped` sequences".]**

said hairpin structure, has an energy lower than -25 Kcal/mole, as predicted by a use of an MFOLD algorithm version 3.7x with default parameters, **[Fig. 12B, paragraphs 226 "...MFOLD algorithm", 230 "...a maximal threshold for free energy"; Program Listing (file: HAIRPIN_PREDICTION.txt, functions: run_palgrade, get_palgrade, get_this_grade, get_nobulge_score; parameters: model_params); and Sequence Listing.]**

said microRNA precursor nucleotide sequence forms part of a polynucleotide sequence forming part of the human genome, said polynucleotide sequence having a plurality of different base pair arrangements which are predictable by a use of said MFOLD algorithm, most of said arrangements including

said hairpin structure, **[Fig. 8, paragraph 178 "...is encoded by the human genome"; Fig. 12B, paragraphs 226, 231-233 "...in at least a selected majority of the alternative secondary structures folding patterns"; Program Listing (file: HAIRPIN_PREDICTION.txt, functions: run_palgrade); and Sequence Listing.]**

at least 70% of nucleotides of a 5' arm of said hairpin structure and of a 3' arm of said hairpin structure are base paired, **[Fig. 12B, paragraph 228: "The HAIRPIN DETECTOR 114 then assesses the hairpin structures found by the previous step, comparing them to hairpin structures of known miRNA precursors, using various characteristic hairpin structure features... Only hairpins that bear statistically significant resemblance to the training set of hairpin structures of known miRNA precursors, according to the abovementioned parameters, are accepted.", "amount and type of mismatched nucleotides"; Fig. 12B, paragraph 228: "amount and type of mismatched nucleotides"; Program Listing (file: HAIRPIN_PREDICTION.txt, function: get_nobulge_score); and Sequence Listing.]**

said microRNA precursor nucleotide sequence includes at least 19% Adenosine nucleotides, at least 16% Cytosine nucleotides, at least 23% Thymine nucleotides and at least 19% Guanine nucleotides, and **[Fig. 13C paragraph 258: "the nucleotide content of the diced miRNA and/or of the hairpin shaped miRNA precursor"; Sequence Listing; and Program Listing (file: HAIRPIN_PREDICTION.txt, function: get_let_scores_exp).]**

Shanon Entropy of a single nucleotide distribution in any 10 nucleotide long portion of said microRNA precursor nucleotide sequence is at least 0.7 bits; **[Fig. 12B, paragraph 228: "...existence of sequence repeat-elements"; Program Listing (file: HAIRPIN_PREDICTION.txt, function: get_seq_complexity); and Sequence Listing.]**

said microRNA nucleotide sequence being further characterized in that:

a number of base paired nucleotides located between said microRNA nucleotide sequence and a loop of said hairpin structure is greater than 1 and smaller than 7, **[Fig. 13B, paragraph 240: "A general goal of the DICER-CUT LOCATION DETECTOR TRAINING &VALIDATION FUNCTIONALITY 126**

is to analyze the dicer-cut locations of known diced miRNA on respective hairpin shaped miRNA precursors in order to determine a common pattern in these locations, which can be used to predict dicer cut locations on GAM folded precursor RNAs."; Fig. 13C, paragraph 257: "represented by their respective distances from the loop"; Program Listing (file: TWO_PHASED_PREDICTOR.txt, function: p_loopdist); and Sequence Listing.]

a number of base paired nucleotides comprised in said microRNA nucleotide sequence is greater than 14, **[Fig. 13B, paragraphs 240 (see above), 241: "...locations of one or more bound nucleotide pairs"; Program Listing (file: TWO_PHASED_PREDICTOR.txt, function: num_bps_prob_hist); and Sequence Listing.]**

with respect to a microRNA complement nucleotide sequence, which is a nucleotide sequence comprised in said microRNA precursor nucleotide sequence, is located opposite said microRNA nucleotide sequence on said hairpin structure and is partially reverse complementary to said microRNA nucleotide sequence, an absolute value of a difference between the number of unpaired nucleotides in said microRNA nucleotide sequence and the number of unpaired nucleotides in said microRNA complement nucleotide sequence is smaller than 3; and **[Fig. 13B, paragraphs 240 (see above), 241: "...locations of one or more mismatched nucleotide pairs"; Sequence Listing; and Program Listing (file: TWO_PHASED_PREDICTOR.txt, function: win_sym_prob).]**

said microRNA nucleotide sequence constitutes less than 0.1% of size fractionated RNA expressed in human HeLa cells, which size fractionated RNA is approximately 20 nucleotides in length. **[All 23 prior art human microRNAs (Tuschl, Science, 294, 853-858 (2001)), were cloned and sequenced from size fractionated RNA from human Hela cells.]**

66. (New) A microRNA oligonucleotide according to claim 65 and wherein said microRNA precursor sequence excludes oligonucleotides consisting solely of nucleotide sequences selected from the group consisting of accession numbers MI0000073- MI0000091, MI0000263-MI0000266, MI0000437, MI0000466-MI0000468 and MI0000651.

67. A microRNA oligonucleotide according to claim 65 and wherein said microRNA oligonucleotide is not expressed in human HeLA cells.

68. A microRNA oligonucleotide according to claim 65 and wherein said microRNA oligonucleotide does not have a mouse homolog.

Claims 1-64 have been cancelled without prejudice.

Applicants express their appreciation to SPE Dr. Michael Woodward and Examiner Dr. Ardin H. Marschel for the courtesy of an interview which was granted to applicant and applicants' representative, Sanford T. Colb (Reg. No. 26,856). The interview was held at the USPTO on June 2, 2004.

During the interview, the application was discussed in general, and there was some discussion concerning clarifying the function of the microRNA oligonucleotides of the present invention. The Applicant respectfully submits the attached appendix containing discussion clarifying the function of the predicted microRNA genes of the present invention.

In view of the foregoing remarks, all of the claims are believed to be in condition for allowance. Favorable reconsideration and allowance of the application is respectfully requested.

Respectfully submitted,

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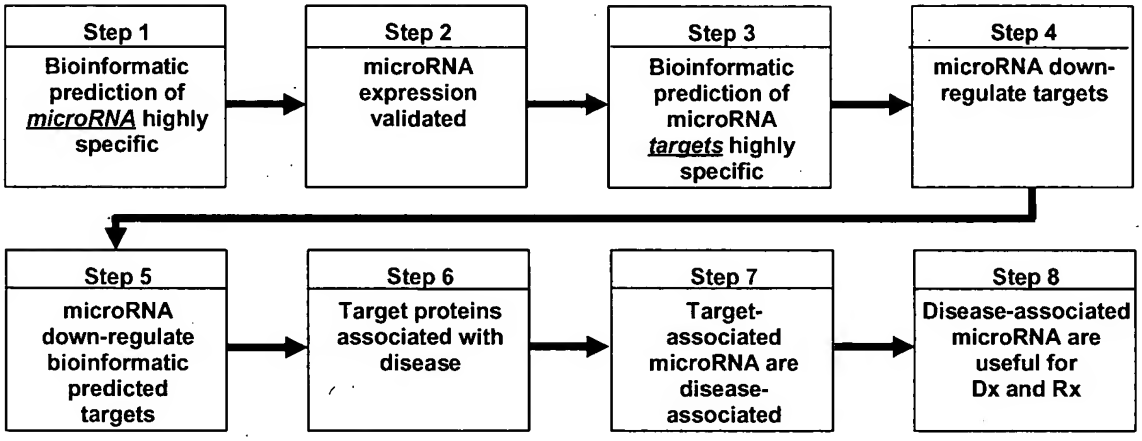
Mitchell B. Wasson
Reg. No. 27,408
HOFFMAN, WASSON & GITLER, P.C.
2461 South Clark Street, Suite 522
Crystal Center 2
Arlington, VA 22202
(703) 415-0100

UTILITY OF OLIGONUCLEOTIDES OF THE PRESENT INVENTION

SUMMARY

The present invention discloses a specific, substantial and credible utility for each of the microRNA oligonucleotides of the present invention (referred to in the specification as GAM oligonucleotides), in a succession of 8 logical steps: (1) bioinformatic prediction of microRNAs provided by the present invention is highly specific; (2) expression of several predicted microRNAs has been lab-validated; (3) bioinformatic prediction of microRNA targets provided by the present invention is also highly specific; (4) microRNAs are known in the art to inhibit translation of their target genes[1-12]; (5) published lab-studies have shown that microRNAs predicted bioinformatically to inhibit translation of respective target proteins, indeed inhibit these target proteins[13]; (6) target proteins of microRNAs of the present invention are known in the art to be associated with diseases; (7) by inference, a microRNA which regulates a disease-associated target, is itself disease-associated; and (8) a microRNA associated with a disease may be used as a diagnostic marker for that disease[14-18]. These steps are schematically illustrated below.

The following pages provide a detailed description of the abovementioned logical steps, an example of the function and utility of a specific microRNA of the present invention using these steps and a listing of cited references.



DETAILED DESCRIPTION OF LOGICAL STEPS

1. **Bioinformatic prediction of microRNAs is highly specific:** The bioinformatic detection system of the present invention accurately detects ~70% of the training-set of published microRNAs, while filtering out ~88.5% of the candidate predictions (Figs 21A and 21B).
2. **MicroRNA is actually expressed:** Expression of 43 novel bioinformatically predicted microRNAs of the present invention was validated by laboratory experiments (Figs. 22A-24D, thus validating the efficacy of the bioinformatic microRNA detection of the present invention.
3. **Bioinformatic prediction of microRNA targets is highly specific:** Using stringent detection settings[19], the bioinformatic microRNA target prediction system of the present invention accurately identifies 14 of the 20 published microRNA binding sites, and identifies an average of only 28 (!) suitable targets for microRNAs of the present invention, out of ~5,700 possible disease-related protein-coding targets checked (Figs 14A and 14B).

4. **Published microRNAs down-regulate their targets:** Numerous studies have fully established that microRNAs specifically inhibit translation of respective protein-coding target genes by binding to partially complementary binding site sequences in the untranslated regions (UTRs) of the mRNA of these target genes[1-12]. Elaborate point-mutation studies have shown the high specificity of microRNA binding sites required for their activity[8].

5. **MicroRNAs down-regulate bioinformatically-predicted targets:** In a recent study[13], 11 out of 15 bioinformatically-predicted target genes of human microRNAs were shown experimentally to indeed be down-regulated by these microRNAs. Every one of these 11 validated microRNA targets is accurately predicted by the microRNA target prediction algorithm of the present invention.

6. **Target proteins are associated with a disease:** MicroRNA target proteins (i.e. proteins whose expression is modulated by a microRNA) of the present invention are known in the art to be associated with diseases. The specification provide, for each microRNA target protein of the present invention, an elaborate summary of extensive published laboratory studies establishing its association with one or more diseases (Tables 8 and 9).

7. **A microRNA which regulates a disease-associated target, is disease-associated:** It is appreciated that a microRNA which regulates expression of a disease-associated target, is itself associated with that disease.

8. **A microRNA associated with a disease may be used for diagnosis and therapy:** The specification describes different modes of diagnosis and therapy for disease-associated microRNAs of the present invention (Figs. 19, 20A and 20B), e.g. paragraph 294: "It is appreciated that since GAM oligonucleotides...modulate expression of disease related target genes, that detection of expression of GAM oligonucleotides in clinical scenarios associate with said diseases is a specific, substantial and credible utility." Numerous studies have suggested a role for various microRNAs in different diseases, including various forms of cancer such as chronic lymphocytic leukemia[14], aggressive B-cell leukemia[15], and colorectal neoplasia[17]. In a recent review entitled "MicroRNAs and Cancer"[16], Dr. M.T.McManus of MIT states: "Hopefully, at least some of these studies will lead to mouse models for study of human diseases, and RNA-based therapies can be tested which counteract the post-transcriptional misregulation of gene expression."

EXAMPLE: FUNCTION AND UTILITY OF GAM1863

An example may be useful to illustrate the abovementioned logical steps. GAM1863 is a novel bioinformatically predicted microRNA of the present invention. The sequence of GAM1863, and its precursor secondary structure are described in Tables 1 and 4 respectively, and are highly similar in pattern to those of published microRNAs (step 1), Table 3 provides its exact genomic location. Lab-experiments validate the efficacy of these bioinformatic predictions (step 2).

TABLE 1: SEQUENCE OF GAM1863: TGCTAGGTTTTTCCTTTCCCC

TABLE 4: HAIRPIN STRUCTURE OF GAM1863 PRECURSOR:

```
     TATA      T   ----------          CCT---        C-   TC
TTCAG    GCAAGG GCT          AGGTTTTTT       TTCCCCA  CTG  T
AAGTC    TGTTCC CGG          TCCGAAGAG       AAGGGGT  GAT  /
     CAG-      -   TGGGTAAGAT          TAAATT        CC   TC
```

TABLE 7: BINDING SITES OF GAM1863 ON 3'UTR OF 'BACE'

```
       CT       -
      TG  AGGTTTTT TCCTTTCCC
      AC  TTCAGGAG GGGAAAGGG
        AC       T

            G          TC
      TGCTAG TTTTTTCCTT  CC
      ACGGTC GGAAAAGGAA  GG
             -          GA
```

Table 7 specifies two adjacent binding sites of GAM1863 in the 3'UTR of "beta-site APP-cleaving enzyme" (BACE), both of which are highly similar in free-energy, nucleotide-pairing pattern

and secondary structure to binding sites of published microRNAs (step 3). A function of GAM1863 is therefore inhibition of BACE (steps 4 & 5).

BACE, a target of GAM1863, is associated with Alzheimer Disease (Alzheimer). Table 8 provides a detailed summary of published laboratory and animal studies validating the association of BACE with Alzheimer: "...By in situ hybridization, expression of BACE mRNA in rat brain was observed at higher levels in neurons than in glia, supporting the idea that neurons are the primary source of the extra cellular A beta deposited in amyloid plaques. ...BACE, a GAM1863 target gene, is responsible for the proteolytic processing of the amyloid precursor protein and therefore is associated with Alzheimer". Table 9 cites additional studies[18,20-28] establishing the role of BACE in Alzheimer (step 6). The full section of Table 8 describing functions of BACE and its association with Alzheimer is attached as an annex hereinbelow.

It therefore follows that GAM1863 is associated with Alzheimer. Under-expression of GAM1863 may lower the level of GAM1863's translational inhibition of BACE. This may lead to an over-expression of BACE, which would increase β-secretase activity, leading to the protelytic cleavage of amyloid precursor protein and the generation of amyloid beta peptide[28]. The deposition of amyloid beta peptide is an early and critical feature of Alzheimer. Thus, under-expression of GAM1863 may lead to an over-expression of BACE, which is known in the art to contribute to the pathogenesis of Alzheimer[26,28] (step 7).

Accordingly, detection of level of expression of GAM1863, may be useful in the diagnosis and staging of Alzheimer and the study of its pathogenesis. GAM1863 may also be useful for therapeutic purposes relating to Alzheimer: in order to lower pathologically high cellular levels of BACE, cells may be transfected with an artificial DNA molecule encoding a GAM1863 which inhibits translation of BACE (step 8).

ANNEX: FUNCTION AND UTILITY OF GAM1863 - ASSOCIATION OF 'BACE' WITH ALZHEIMER (FROM TABLE 8)

"Beta-site APP-cleaving Enzyme (BACE, Accession NM_012104.2) is another GAM1863 target gene. BACE BINDING SITE1 and BACE BINDING SITE2 are target binding sites found in untranslated regions of multiple transcripts of mRNA encoded by BACE, corresponding to target binding sites such as BINDING SITE I, BINDING SITE II or BINDING SITE III of Fig.8. Nucleotide sequences of BACE BINDING SITE1 and BACE BINDING SITE2, and secondary structure complementary to the nucleotide sequence of GAM1863 RNA are set forth in Tables 6 and 7, hereby incorporated herein.

Another function of GAM1863 is therefore inhibition of BACE, a GAM1863 target gene which is responsible for the proteolytic processing of the amyloid precursor protein and therefore is associated with Alzheimer. Accordingly, utilities of GAM1863 include diagnosis, prevention and treatment of Alzheimer, and of other diseases and clinical conditions associated with BACE. The function of BACE and its association with various diseases and clinical conditions, has been established by previous studies, as described hereinabove with reference to GAM99."

"The function of BACE has been established by previous studies. Cerebral deposition of amyloid beta peptide is an early and critical feature of Alzheimer (104300). Amyloid beta generation depends on proteolytic cleavage of amyloid precursor protein (APP; 104760) by 2 proteases, beta-secretase and gamma-secretase. Vassar et al. (1999) reported the cloning of a human trans membrane aspartic protease that had all the known

characteristics of the beta-secretase. Using an expression cloning strategy, they identified a clone that shared significant sequence similarity with members of the pepsin subfamily of aspartic proteases. This clone encoded a novel protein, designated BACE for 'beta-site APP-cleaving enzyme.' The BACE open reading frame encodes a protein of 501 amino acids containing a 21-amino acid signal peptide followed by a proprotein domain spanning amino acids 22 to 45. The lumenal domain of the mature protein is followed by 1 predicted trans membrane domain and a short cytosolic C-terminal tail of 24 amino acids. BACE was predicted to be a type 1 trans membrane protein with the active site on the lumenal side of the membrane, where beta-secretase cleaves APP. The BACE protein shares greatest amino acid identity (30%) with cathepsin E (OMIM Ref. No. 116890). Rat and mouse BACE orthologs have 96% amino acid sequence identity with the human BACE protein. Northern blot analysis of human BACE mRNA in adult peripheral tissues and various sub regions of the brain detected 3 transcripts of approximately 7.0, 4.4, and 2.6 kb. By in situ hybridization, expression of BACE mRNA in rat brain was observed at higher levels in neurons than in glia, supporting the idea that neurons are the primary source of the extra cellular A- beta deposited in amyloid plaques. Vassar et al. (1999) ascribed the difference between the apparent and calculated molecular weight (approximately 70 and 51 kD, respectively) of the BACE protein to N-linked glycosylation. Immunostaining demonstrated intracellular localization of BACE to the Golgi and endosomes. Transient overexpression of BACE did not affect APP expression, but decreased alpha-secretase cleavage and increased beta-secretase activity in cells expressing wildtype or Swedish mutant (104760.0008) APP. BACE overexpression induced cleavage only at the known beta-secretase positions, asp1 and glu11. Vassar et al. (1999) concluded that their data provided strong evidence that the BACE aspartic protease is the long-sought beta-secretase.

Animal model experiments lend further support to the function of BACE. Luo et al. (2001) found that mice deficient in BACE1 are healthy, fertile, and appear normal in gross anatomy, tissue histology, hematology, and clinical chemistry. Bace1 -/-mice who are also hemizygous for an amyloid precursor protein transgene lack brain beta-amyloid and beta-secretase-cleaved APP C-terminal fragments. These results provided validation of BACE1 as the major beta-secretase in vivo and suggested that therapeutic inhibition of BACE1 for the treatment of Alzheimer may be free of mechanism-based toxicity. It is appreciated that the abovementioned animal model for BACE is acknowledged by those skilled in the art as a scientifically valid animal model, as can be further appreciated from the publications sited hereinbelow.

Full details of the abovementioned studies are described in the following publications, the disclosure of which are hereby incorporated by reference:

Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, S.; Mendiaz, E. A.; Dents, P.; Taplow, D. B.; Ross, S.; Amaranta, P.; Loeloff, R.; Luo, Y.; Fisher, S.; and 12 others : Beta-secretase cleavage of Alzheimer amyloid precursor protein by the trans membrane aspartic protease BACE. Science 286: 735-741, 1999. ; and

Luo, Y.; Bolon, B.; Kahn, S.; Bennett, B. D.; Babu-Khan, S.;Denis, P.; Fan, W.; Kha, H.; Zhang, J.; Gong, Y.; Martin, L.; Louis, J.-C.; Yan, Q.; Richards, W. G.; Citron, M.; Vassar, R.

Further studies establishing the function and utilities of BACE are found in John Hopkins OMIM database record ID 604252, and in cited publications listed in Table 9, hereby incorporated herein."

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